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The brain α7 nicotinic receptor may be an important therapeutic target for the treatment of Alzheimer's disease: studies with DMXBA (GTS-21)

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Abstract

A large decrease in brain nicotinic receptor levels occurs in Alzheimer's disease, relative to muscarinic and other receptors. Neurons possessing high affinity nicotinic receptors seem particularly vulnerable. The low affinity nicotinic receptors which selectively bind α-bungarotoxin are not significantly affected. The major nicotinic receptor subtype which binds this toxin is a homo-oligomer composed of α7 subunits. Due to its exceptionally high calcium ion selectivity, this particular receptor can be considered as a ligand-gated calcium channel. α7 receptors are found in regions of the brain which are important for cognition, including cerebral cortex and hippocampus. Hippocampal receptors are largely confined to GABAergic interneurons. α7 receptors seem less likely than α4–β2 receptors to be up-regulated in number and down-regulated in function as a result of chronic agonist exposure. A family of nicotinic agonists based upon the marine animal toxin anabaseine have been synthesized and investigated. One of these compounds, DMXBA [3-(2,4-dimethoxybenzylidene)-anabaseine; code name GTS-21] has displayed promising characteristics during phase I clinical tests. In the rat DMXBA is selectively agonistic upon α7 nicotinic receptors. In addition it is a moderately potent antagonist at α4–β2 receptors. DMXBA enhances a variety of cognitive behaviors in mice, monkeys, rats and rabbits. It also displays neuroprotective activity upon cultured neuronal cells exposed to β-amyloid or deprived of NGF. The compound is much less toxic than nicotine and does not affect autonomic and skeletal muscle systems at doses which enhance cognitive behavior. Phase I clinical tests indicate that large doses can be safely administered orally without adverse effects. Psychological tests on healthy young male subjects indicate a positive effect of DMXBA on some measures of cognition. While DMXBA is a much weaker partial agonist on human α7 receptors than upon rat α7 receptors, its 4-hydroxy metabolite has been shown to have excellent efficacy on both receptors. Thus, some of the physiological and behavioral effects of GTS-21 may be due to the actions of this primary metabolite.

Keywords: α7 Nicotinic receptor; Alzheimer's dementia; Anabaseine; Cognition; GTS-21

1. Introduction

Cholinergic innervation of the cerebral cortex and hippocampus is particularly vulnerable to disruption in Alzheimer's disease (AD). There is a similar decrease in choline acetyltransferase, high affinity nicotinic receptor binding, and choline transporter sites in AD post-mortem brains [50,60,64,69]. Whether the loss of these nicotinic receptors represents a primary lesion in the disease or simply occurs because these receptors may be largely localized on vulnerable neurons has not yet been completely resolved, but the concomitant loss of the other cholinergic components of the nerve terminal suggest that the latter possibility is the case. These results, along with the demonstration of cognitive behavioral deficits in animals treated with cholinergic antagonists and in lesioned animals, led to the formulation of the so-called 'cholinergic hypothesis', which emphasizes the consequences of preferential degeneration of the cholinergic projecting neurons in Alzheimer's dementia. A therapeutic corollary of this

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hypothesis is that treatments which reduce this cholinergic degeneration or counteract the resulting cholinergic hypofunction should be useful. Historically, this led first to the development of acetylcholinesterase inhibitors, which only in the past few years have been shown to provide some limited symptomatic benefit to AD patients. The second phase in cholinergic drug design for AD focused upon muscarinic receptor agonists, particularly M1 agonists, since muscarinic receptors are much more numerous (> 50–100X) in the mammalian brain than are nicotinic receptors. Considerable interest in CNS nicotinic receptors developed when several laboratories almost simultaneously [6,50,60,64,69] reported drastic (≥ 50%) losses in cerebral cortex high affinity nicotinic receptors in AD, while changes in muscarinic receptors were relatively quite small [69]. Thus, CNS nicotinic receptors may be drug targets for treatment of AD and a variety of other disorders [14,33,38,60].

In the past 5 years several nicotinic drug candidates have entered clinical tests for potential use in the treatment of AD. These may be broadly classified into two groups, based upon the particular type of nicotinic receptor subtype that is the drug's target. The initial drug group, including ABT-418 and nicotine, primarily targets nicotinic receptors containing the β2 subunit, of which α4–β2 is most abundant. The second group of drugs primarily target the predominant low affinity subtype, the α7 receptor. Stimulation of either receptor type has been shown to enhance cognitive behaviors in experimental animals. This article will focus upon the α7 nicotinic receptor as an AD drug target, summarize the therapeutic rationale for selecting this receptor target and present the current understanding of the properties of DMXBA (GTS-21), the first α7 nicotinic agonist drug candidate to be developed.

2. Rationale for developing α7 agonists to treat AD

The theoretical basis for using this nicotinic receptor subtype as an AD therapeutic target is summarized as follows:

1. The α7 receptor, unlike high affinity nicotine receptors, does not disappear as AD progresses [13,44,60,64]. Thus the drug target remains able to respond to stimulation.
2. The α7 receptor channel is highly permeable to the calcium ion. Calcium acts as a second messenger inside the neuron and not only stimulates neurotransmitter, but also stimulates signal transduction events through stimulation of protein kinases, calcineurins, nitric oxide synthetases and other enzymes.
3. The α7 receptor uniquely mediates the neuroprotective action of nicotinic agonists against various stresses including β-amyloid and nerve growth factor depletion.
4. Stimulation of this receptor enhances cognitive behavior (learning and memory) in a variety of behavioral tasks, even with repeated application.
5. Chronic administration of an agonist (DMXBA) which selectively stimulates this receptor does not lead to significant receptor up-regulation, in contrast to agonists which stimulate the high affinity receptor.
6. DMXBA, and presumably other α7 agonists, do not act as nicotine cues (discriminative stimulus) or possess significant drug dependence (self administration) potential.

Most of these points will be discussed further in the remainder of this article, which will focus upon DMXBA.

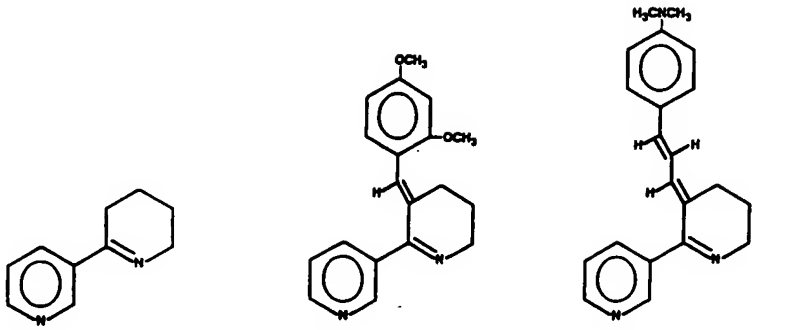

Fig. 1. Chemical structures of Anabaseine, DMXBA (GTS-21) and DMACA.

2.1. Anabaseine analogs as drug candidates

Anabaseine (Fig. 1) is an alkaloid toxin synthesized by a marine worm which utilizes it to paralyze its prey and to avoid predators [33]. It closely resembles another alkaloid, anabasine, which is a major product in the eastern European tobacco plant *Nicotiana glauca*. Anabaseine, and anabasine to some extent, differs pharmacologically from nicotine in possessing a higher efficacy for stimulating the α7 receptor relative to the α4–β2 receptor [32]. However, like nicotine, anabaseine stimulates all known nicotinic receptors to some degree, and thus is also relatively toxic. In the course of studying the chemical and pharmacological properties of anabaseine, the author initially prepared a benzylidene-adduct of anabaseine and found that it lacked neuromuscular and ganglionic stimulatory effects [32]. This led to the synthesis and testing of a wide variety of anabaseine derivatives which will be reported in the near future. From this group a disubstituted benzylidene-anabaseine derivative, DMXBA was selected for further development based upon a variety of preclinical tests. This compound has the code name GTS-21 because it was the 21st compound prepared for the collaborative project between University of Florida scientists in Gainesville and Taiho Pharmaceutical Company scientists in Tokushima, Japan.

DMXBA (Fig. 1) is readily synthesized by condensation of anabaseine with 2,4-dimethoxybenzaldehyde. At low (<1 mg/ml) concentrations the dihydrochloride salt is quite soluble in physiological saline at neutral pH, but for administration of more concentrated aqueous solutions it is necessary to adjust the to pH 6.5 or less, because of the low water solubility of the unionized form. The p*K*a of the imine nitrogen of DMXBA is 7.4 [41]. Thus at normal physiological pH, half of the molecules in solution will be monocations and the other half will be unionized. In contrast with anabaseine, DMXBA does not hydrolyze to an open-chain form in aqueous solution, even at relatively acidic pHs [41,76]. DMXBA is highly lipophilic; the octanol:water partition coefficient of the neutral form is 3800, compared with only 15 for nicotine (Table 3). DMXBA possesses two aromatic rings which are attached to the tetrahydropyridyl ring at adjacent carbons. The anabaseine structure contained within DMXBA provides the basic Beers and Reich minimal nicotinic receptor pharmacophore [32]. However, it is unlikely that the two 'anabaseine' rings within DMXBA retain the coplanar configuration of the toxin, since there is considerable steric hindrance in the region where the two aromatic ring portions of the DMXBA molecule are connected to the tetrahydropyridyl ring [41]. Also, the related compound 3-(4-chlorobenzylidene)-anabaseine was calculated to have a preferred conformation in which all three rings occupy different planes [76]. DMXBA dihydrochloride, like this compound, also exists as the E (entgegen) stereoisomeric form in aqueous solution [41]. However, the Z form can be generated by prolonged exposure to intense light. This form does not readily bind to nicotinic receptors, at least those of the α4–β2 type (Kem and Mahnir, unpublished results). DMXBA dihydrochloride is quite stable when stored in an amber bottle in the anhydrous state.

3. In vitro studies of DMXBA interaction with nicotinic receptors

The *Xenopus* oocyte expression system was instrumental in determining whether DMXBA was an agonist or antagonist, and in delineating its selectivity for particular combinations of receptor subunits. Prior to these experiments it had been established that DMXBA and another benzylidene-anabaseine, DMAB-anabaseine [30], acted as weak antagonists at the neuromuscular junction and on pheochromocytoma cells. A stimulatory action had not yet been found for any nicotinic receptor. α7 receptor stimulation is difficult to demonstrate because of rapid desensitization and its absence in many in vitro preparations. DMXBA and DMACA, were shown to behave as partial agonists on the rat α7 receptor expressed in the *Xenopus* oocyte [15,54]. DMACA was a more efficacious as well as potent agonist upon this receptor. A large variety of benzylidene- and cinnamylidene-anabaseines have been made in this laboratory over the past 12 years. This has led to the identification of several compounds possessing some PK and PD advantages over DMXBA and DMACA. These have now become lead compounds for the development of more refined drug candidates (Kem et al., unpublished results).

In the oocyte system both DMXBA and DMAC-anabaseine generate currents that more slowly develop and decay with continued bath application relative to ACh [15,54]. Thus, even though they produce a smaller maximal peak current response than ACh, the total current carried is not so small, relative to the maximal peak current. Measurements of the residual inhibition of the response to a second ACh application 5 min after washing away the compound also indicated that the inhibitory effects of these two compounds washed away relatively slowly. This could be caused by several possible factors, alone or in concert. At the receptor level there could be use-dependent inhibition caused by ion channel blockade or a prolonged desensitization. It is also possible that the washout of these very lipophilic compounds from the oocyte is a very slow process. Further analysis is required to determine the basis for this inhibition.

Both the Abbott and University of Florida groups have examined the effect of DMXBA upon the human form of the α7 receptor and found that DMXBA is much less efficaceous and potent than upon the rat receptor. On the other hand, the UF group has found that the major phase I metabolite, 3-(4-hydroxy-2-methoxybenzylidene)-anabaseine, is just as good on the human receptor as is DMXBA on the rat receptor. These two compounds have essentially identical efficacies on the rat receptor [41,47]. The explanation for this species difference is not yet known, but the fact that the human receptor ACh recognition site possesses a phenylalanyl side chain at a critical position (# 222) instead of the tyrosyl side chain present in the other vertebrate α7 receptor forms suggests that this position may be critical [47]. Regardless of the underlying mechanism, it clearly becomes of the utmost importance to test new compounds on the human form before considering them as serious drug candidates. In contrast, the other major receptor, α4–β2, has not yet exhibited any important species differences in its responsiveness to DMXBA and other nicotinic ligands [11].

The antagonistic effects of DMXBA upon rat and human α7 nicotinic receptors have also been demonstrated [9,15,54]. It has been known for many years that synchronous nicotinic receptor activation displays abiphasic time course, an initial stimulation being followed by inhibition of the receptor. However, at subthreshold concentrations, nicotine, DMXBA and many other nicotinoids can act as antagonists without causing any noticeable initial depolarizing current. This does not eliminate the possibility that similarly low concentrations of these agonists may be capable of generating calcium currents that would exert tonic, second messenger effects. One of the major challenges in establishing a scientific rationale for the use of nicotinic agonists is to delineate a physiological mechanism for α7 stimulation in the presence of very low concentrations of a nicotinic agonist.

Synaptic transmission occurs as a result of pre- or postsynaptic α7 receptor stimulation at some synapses [12,22,24,26,76]. Until now, few studies have been carried out on the effects of DMXBA upon the endogenous nicotinic receptors in brain neurons. A potentiating effect of this compound upon long-term potentiation in hippocampal synaptic transmission over a very narrow concentration range, was initially reported several years ago [28]. However, subsequent attempts to confirm the existence of this LTP enhancement were unsuccessful (B. Hunter, personal communication). At present, there is no strong evidence for nicotinic receptor involvement in hippocampal LTP. Since α7 receptors in the hippocampus primarily modulate GABAergic rather than glutamatergic transmission this is not altogether surprising.

One of the most vexing questions in the central nicotinc receptor field is how relatively constant agonist concentrations are capable of enhancing cognition and presumably, the neuronal pathways mediating the effects. Until recently the peripheral nervous system paradigm of nicotinic receptor function was considered to be applicable towards explaining the functional roles of central receptors. At both the skeletal muscle synapse and at ganglionic synapses nicotine can be demonstrated to produce phasic depolarizations of the postjunctional membrane which are sufficient to trigger the electrical activation of the muscle or postganglionic neuron. The problem of extrapolating from this extensive peripheral system knowledge to the CNS is particularly acute for the α7 nicotinic receptor, since it desensitizes more rapidly than the other neuronal receptors. Almost all functional studies of this receptor have monitored peak responses to rapid applications of the nicotinic agonist, as in *Xenopus* oocyte experiments. However, this does not reflect the physiological situation which would occur in an Alzheimer's patient where the nicotinic agonist levels would be maintained relatively constant over periods of hours or days. It is possible that at extremely low GTS-21 concentrations there is a relatively small, but non-desensitizing Ca influx through the small fraction of α7 channels that are occupied which is sufficient to enhance synaptic function and cognition. In this regard, it has been recently reported that some mammalian α7 receptors desensitize more slowly than others [12], and that the degree of desensitization depends upon agonist concentration [59].

Radcliffe and Dani [57] have shown that nicotine produces long-lasting as well as almost instantaneous enhancement of glutamatergic transmission. The long-lasting effect was suggested to be a result of a second messenger action of calcium ions entering the neuron through the nicotinic receptor channel, perhaps affecting protein kinases. In the past few years it has been shown that nicotinic stimulation causes the release of nitric oxide a chemical which can readily diffuse to a wider region of neuronal tissue than do conventional neurotransmitters. Thus it seems possible that nicotinic agonists may have effects which last much longer than would be expected based on the peripheral NS paradigm.

The agonist properties of anabaseine, nicotine and DMXBA are summarized in Table 1.

4. Effects on other receptors

A limited number of neurotransmitter (5HT1A, 5HT2A, adenosine2A adenosine1, muscarinic (QNB binding), NMDA-glutamate, and total glutamate receptors) and voltage-gated ion channel (brain Kv1 channel

Table 1
Comparison of the relative efficacies of anabaseine, nicotine and DMXBA action upon the major nicotinic receptors

Receptor type	Anabaseine	Nicotine	GTS-21
Central:			
α7 (Rat)	Full agonist	Partial agonist	Partial agonist
α4–β2 (Rat)	Partial agonist	Partial agonist	Antagonist
Peripheral:			
α3–β4 (Rat PC12)	Full agonist	Full agonist	Weak antagonist
Skeletal muscle (Frog or mouse)	Full agonist	Full agonist	Weak antagonist

Table 2
Effects of DMXBA upon behavioral tasks measuring cognition

Behavioral assay	Effect ($P<0.05$)	Ref.
Passive avoidance (les. Rat)	++	[45]
Passive avoidance (mouse)	0	[14]
Active avoidance (aged rat)	++	[3]
Lashley III maze (aged rat)	++	[3]
Morris water maze (aged rat)	++	[48]
11-Arm radial maze (aged rat)	++	[3]
Eyeblink conditioning (aged rabbit)	++	[72]
Delayed matching task (monkey)	++	[10]

hetero-oligomers, L-type Ca) receptors have been screened by radioligand binding methods to assess the selectivity of DMXBA. It only affected some of these receptors at very high concentrations (> 50 μmol), which are at least 100 times higher than the plasma concentrations observed at doses used for behavioral testing. A 71% inhibition of brain L-type Ca channels was observed at 50 μmol diltiazem, but it is doubtful if this interaction would be of physiological significance because of the relatively high DMXBA concentrations which would be required to inhibit most L-type channels.

A potentially significant interaction of DMXBA was observed with 5-HT_3 receptors was found by electrophysiological recordings from the *Xenopus* oocyte expression system. DMXBA acted as a weak antagonist on the mouse receptor, but the monohydroxy metabolites were partial agonists of differing potency [39,27]. A more limited set of experiments have been carried out using the human form of this receptor, and in this case all of the compounds behaved as weak antagonists (Machu et al., unpublished results). Unfortunately, the effects of 5-HT_3 receptor agonists upon neurotransmitter levels and cognition are not well understood at this time.

DMXBA also inhibits rat brain acetylcholinesterase, but only at very high concentrations (> 20 μM) which are unlikely to be attained therapeutically (Kem, unpublished results).

4.1. Behavioral actions: cognitive behavior

The effects of 1–10 mg/kg doses of DMXBA upon a variety of cognitive behaviors have been investigated upon four mammalian species at several other universities and pharmaceutical companies, including two pharmaceutical companies (Abbott Pharmaceuticals in the US and Synthelabo, France) who independently synthesized and tested DMXBA. Considering that there were differences in the behavioral protocols, the consistency of the results are rather impressive (Table 2).

While active and passive avoidance tests are not the most sophisticated tests of cognition, their speed and simplicity are useful in the initial screening of a series of compounds. In the initial DMXBA tests nucleus basalis magnocellularis lesioned animals were used as previously described [45,48]. Aged rats without lesions were also used for active avoidance tests [3,8]. More recently, cholinergic hypofunction was induced by scopolamine [29]. In all of these tests i.p. DMXBA was found to increase passive (measured at 24 or 72 h post training) and active avoidance at doses only slightly greater than were equally effective for nicotine.

A more elaborate behavioral analysis was also carried out by Dr Woodruff-Pak's laboratory at Temple University using the well-developed eye blink conditioning system with aged female rabbits [70]. While this behavior does not depend upon an intact hippocampus, it is greatly affected by alterations in hippocampal function. One of its major advantages is its quantitative precision. Another advantage is that the testing paradigm can be used with humans as well; in fact, AD patients show a significant decrease in rate of acquisition of conditioned responses. It was initially established that the non-competitive nicotinic antagonist mecamylamine inhibited acquisition of the conditioned response without affecting the response to the unconditioned response, at a dose which would not be expected to block NMDA-type glutamate channels [71]. Over a 2-week period three different doses of DMXBA were tested and a dose-dependent enhancement of the rate of acquisition was demonstrated (Fig. 2). In fact, at the highest dose the aged rabbits learned the conditioned response as readily as did young female rabbits. It was also found that the concentration of high affinity and later α7 receptors is unchanged after daily DMXBA administration for these tests [72].

Effects of DMXBA upon spatial memory tasks were investigated using the Morris water maze as well as the radial maze. DMXBA enhanced working as well as reference memory [3,8]. The Abbott paper [10] also

presents data by the Buccafusco laboratory demonstrating DMXBA enhancement of delayed matching performance in monkeys.

Evidence for involvement of hippocampal nicotinic high affinity and low affinity (α7) receptors in the effects of nicotine upon spatial memory has been reported by Felix and Levin [18].

4.2. Other behavioral effects of DMXBA: addiction, analgesia and other behaviors?

It has recently been reported that α7 as well as other nicotinic receptors are expressed in ventral tegmental neurons which secrete dopamine in the nucleus accumbens, the site of primary importance for addictive behaviors [36]. Thus the possibility exists that stimulation of α7 receptors may modulate or enhance self-administration and other behaviors such as drug discrimination which contribute to drug addiction.

The action of DMXBA upon nicotine discrimination and self-administration has been investigated. It was found that DMXBA does not act as a nicotine cue or as an inhibitor of the nicotine cue [68]. Administration of DMXBA inhibited self-administration rather than stimulating it, albeit at a rather high dose. The results are thus most readily interpreted at this stage as preliminary evidence that α7 receptors are not major nicotinic receptor contributors to the nicotine cue and of self-administration. However, it must be kept in mind that this compound also is a partial agonist and therefore a partial antagonist at this receptor. In addition, DMXBA is a β2 subunit-containing receptor antagonist as well. Nomikos et al. (Huddinge Symp. Proc.) report that the α7 receptors residing on the nerve terminals of glutamate secreting neurons can enhance stimulation of the VTA dopaminergic neurons, so it is possible that the concurrent inhibition of the VTA high affinity receptors would prevent any stimulatory effect on these glutamatergic neurons from causing DA release. Thus while the mechanistic interpretation may be complex, the fact remains that DMXBA failed to affect this system except to inhibit self-administration at relatively high doses. A smaller study by the Abbott group also indicated minimal or no effects on the nicotine cue [10].

A comparative study of nicotinc agonists by the Abbott group [14] suggested that nicotinic agonist effects upon certain behaviors are largely mediated by the high affinity (α4–β2 and/or α3–β2) receptors rather than by α7 'low affinity' receptors. For instance, DMXBA had no effect upon the hot plate measure of analgesic activity or on the elevated plus-maze measure of anxiolytic activity. The authors note that the hypothermic effectiveness of a particular nicotinic agent can be related to its potency at high affinity nicotinic receptors and its ability to be analgesic. Since DMXBA only affected body temperature at very high doses (24 mg/kg, i.p., rat) and did not display any analgesic activity at this dose, this data suggests that stimulation of α7 receptors is unlikely to have a major influence upon the analgesic and hypothermic pathways stimulated by nicotine. Also, the α7 antagonist MLA did not affect the nicotinic analgesic effect as measured by the tail-flick assay [58].

Nicotine affects the phase-setting of the rodent circadian rhythm, probably by stimulating α7 receptors in the suprachiasmatic nucleus in the hypothalamus [67].

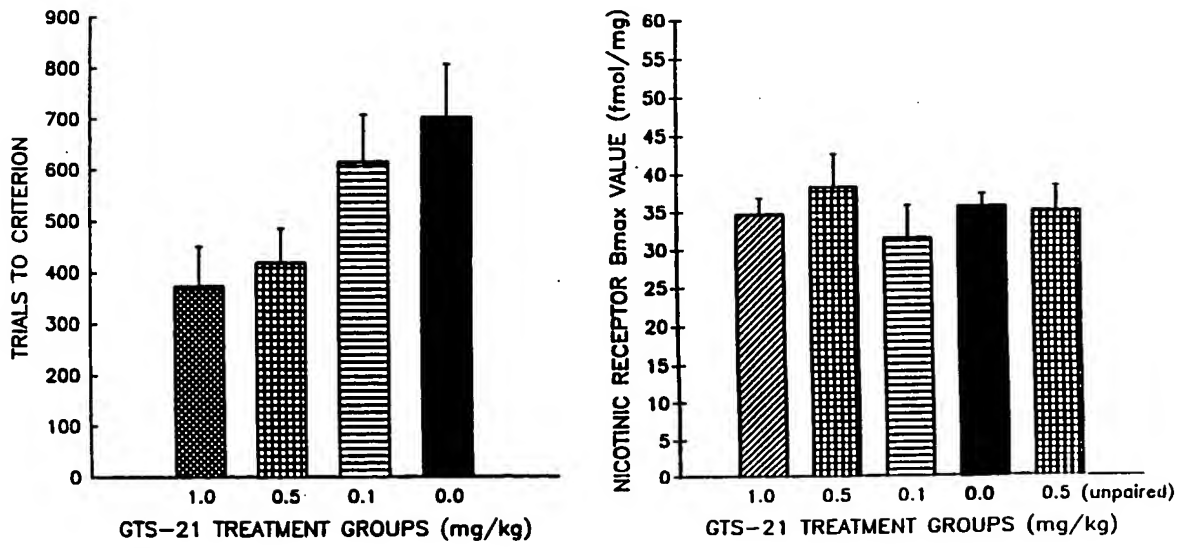

Fig. 2. Enhancement of eyeblink conditioning in aged rabbits and lack of nicotinic receptor up-regulation by DMXBA, from [72]. (A) On the left indicates the number of paired stimulus trials required to learn the eyeblink reflex at three different doses (subcutaneous) of GTS-21, relative to a saline control group ($n = 8$ animals for each group). (B) on the right shows measurements of the concentration of high nicotine affinity receptors in cerebral cortex samples from the same rabbits.

It was recently found that DMABA, a benzylidene-anabaseine which acts similarly to DMXBA [30,33], consistently affects the phase of this daily rhythm in a similar manner [51]. This effect was also observed in vivo in tissue slices of this hypothalamic nucleus, which contains the putative 'clock' cells. Auditory gating deficits in mice can be alleviated by DMXBA [62]. Since this deficit is common in schizophrenia, α7 agonists may be useful drugs for this mental disorder.

5. DMXBA effects upon neurotransmitter levels: microdialysis studies

Giacobini's laboratory in particular has pioneered the use of microdialysis measuements of neurotransmitter level changes as a means of investigating the effects of nicotinic agonists in whole animals [65]. Nicotine, ABT-418 and other agonists which preferentially bind to the β2 subunit-containing receptors stimulate a sizable increase in ACh levels in the prefrontal cortex and hippocampus, while DMXBA (Fig. 3) failed to increase the levels of this NT in either the cortex or the hippocampus [59,66]. This was in contrast with anabaseine, the initial lead compound, which stimulated the release of ACh, norepinephrine, dopamine, but not 5-HT. DMXBA differs pharmacologically from anabaseine in possessing essentially no agonist activity upon any receptor containing B subunits. While DMXBA did elevate the two catecholamines, but not as greatly as did an equal dose of anabaseine or nicotine. The simplest interpretation of the ACh effects would be that β subunit-containing receptors modulate cholinergic neurons while α7 receptors are not a significant population on such neurons. Obviously much more needs to be known about the distribution of nicotinc receptors on projecting neurons secreting these neurotransmitters before the observed actions can be understood. A particularly striking effect of DMXBA occurs after mecamylamine administration; now DMXBA produces a very robust elevation of ACh and 5HT, the two neurotransmitters least affected when it is administered alone [59]. Further experiments are planned to understand some of these initial observations.

5.1. Neuroprotective actions [1,34,37,43,46,61,75]

Intracellular calcium is a double-edged 'sword', stimulating cellular functions at lower concentrations but damaging the cell at excessively high concentrations [11,21]. Ca ions serve as second as well as first messengers under normal physiological conditions [16,19,49]. Under pathological conditions where Ca is greatly elevated, it is an apoptotic stimulus — it stimulates programmed cell death. In the past 5 years several laboratories have shown that nicotine and DMXBA are neuroprotective to a variety of chemical stimuli, including glutamate, aspartate, β-amyloid peptide, and withdrawal of nerve growth factor [1,34,37,43,46,61,75]. In most cases where these studies were carried out with cultured cell lines, it has been demonstrated that the protective actions of these nicotinic compounds can be inhibited by the co-administration of a non-specific nicotinic antagonist such as mecamylamine and or by α7 specific antagonists such as α-bungarotoxin and MLA (note that MLA is not selective at concentrations exceeding 50 nM). Even compounds like ABT-418 [17] which preferentially stimulate high affinity receptors were only blocked under conditions in which the α7 receptors were blocked, leading to the conclusion that α7 receptor stimulation is necessary. None of the nicotinic agonists were active unless they were administered prior to the damaging stimulus, suggesting that they act by allowing some Ca ions to enter the cell and trigger cellular processes which counteract the subsequent Ca overload. Alternately, coadministration might actually cause an ever greater Ca overload. The mechanisms of this nicotinic cytoprotection have not yet been deciphered, and it also needs to be examined in vivo more thoroughly before its clinical potential can be assessed. Nevertheless, it is the major prospect that nicotinic therapy can be more than a treatment of the neurobehavioral symptoms of AD.

5.2. Pharmacokinetics

The most detailed PK characterization of DMXBA has been in the male rat [4,5,40] although dog and monkey data have also been reported [5,10]. One of the most important conclusions is that this compound is very rapidly absorbed after oral administration and quickly enters the brain [5,40]. Both of these findings are understandable based upon its very high octanol:water partition coefficient (Table 3). The absolute bioavailability estimates indicate that approximately one-forth of the orally administered dose entered the systemic circulation, indicating that 'first-pass' intestinal and/or hepatic biotransformation was extensive. Consistent with this interpretation, less than 1% of the initially administered dose was recovered in the urine [5,40]. Using ^{14}C-labeled DMXBA, the Taiho group showed that most of the radioactivity was recovered in the feces, indicating extensive biliary secretion of DMXBA or its metabolites [5]. After intravenous administration, the plasma concentration-time curve displayed bi-phasic kinetics [40] with plasma half-lives of 0.71 and 3.71/h, respectively. The apparent volume of distribution was quite large, indicating drug entry into all aqueous body compartments and binding to peripheral tissues.

The levels of DMXBA in whole brain rather closely followed changes in plasma concentration [5,40]. The whole brain concentration measurements are unlikely to be accurate estimates of the free drug concentration bathing the target neurons in the brain. It would be of

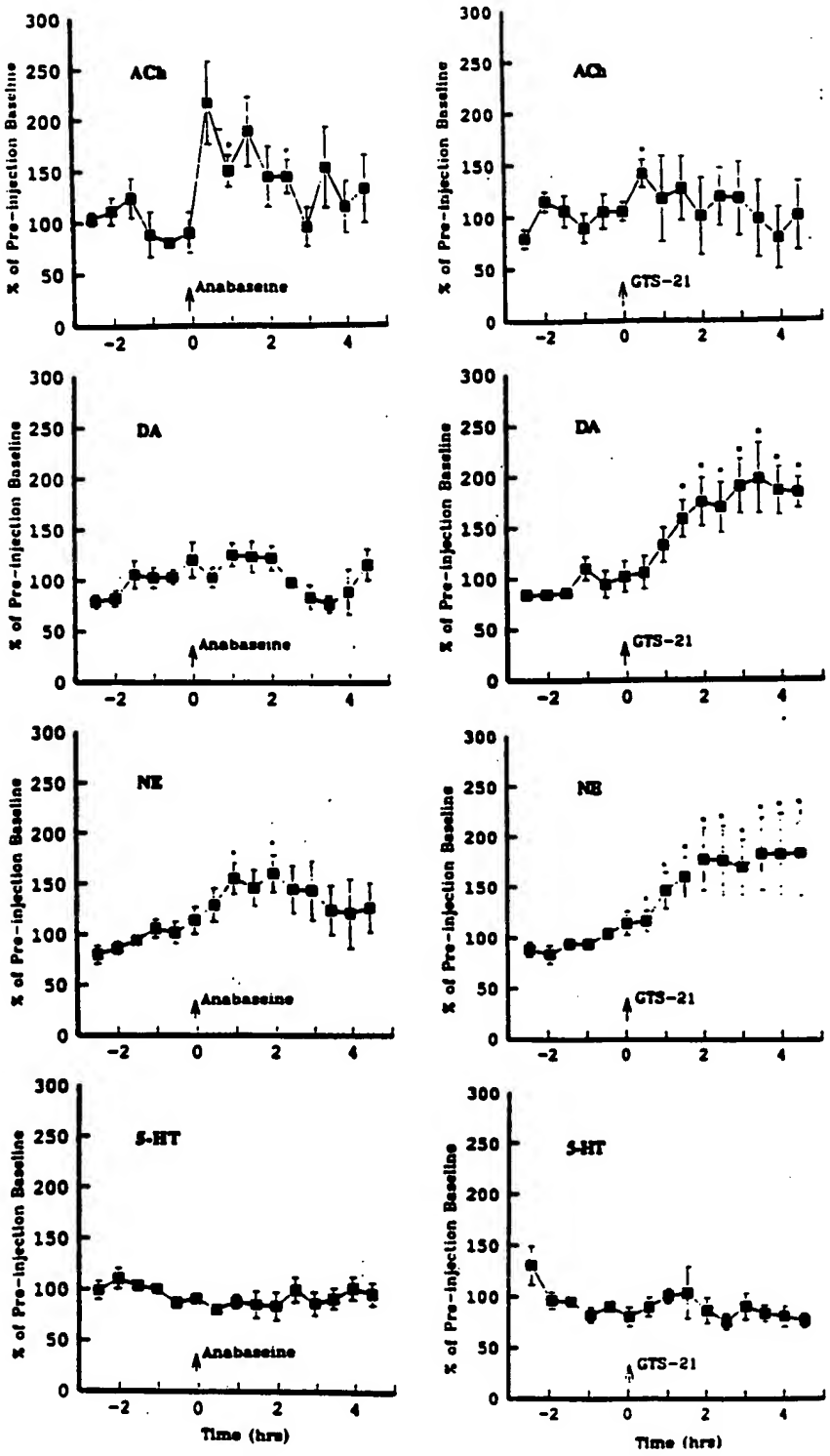

Fig. 3. Microdialysis study of DMXBA effects upon neurotransmitter levels in the rat prefrontal cortex, from [65].

great interpretational value to know the free extracellular concentrations of DMXBA and its metabolites at the doses and times used for the cognitive behavior tests, but thet have not been measured yet. It is intended to use microdialysis methods to estimate these values in the near future.

5.3. Metabolism

Initial hepatic microsome incubation experiments indicated that O-demethylation of both *ortho*- and *para*-methoxy substituents occurred readily. The two monohydroxy and the 2,4-dihydroxybenzylidene

metabolites (Fig. 4) were identified by HPLC-MS comparison with the synthetic compounds [31,41] and by proton NMR [5]. The 4-OH monohydroxy metabolite was produced at approx. twice the rate as the 2-OH monohydroxy metabolite and was the predominant phase I biotransformation product. This also is the case in vivo in rat [31,41], dog and man [4,5]. The particular cytochrome P450s involved have not yet been identified with any certainty, although it appears from human microsome experiments with inhibitors that CYP1A2, 2E1 and CYP3A may be involved [5]. Both human and rat microsome experiments with CY2D6 inhibitors failed to show any evidence for involvement of this cytochrome in the oxidative metabolism of DMXBA [5,41]. Only small amounts of the dihydroxy metabolite were evident in in vivo as well as in vitro experiments [41].

The two monohydroxy metabolites are significantly more potent agonists on rat α7 receptors relative to DMXBA. The 4-OH metabolite was approximately 6 times more potent in this study [31,41] and 8 times more potent in another study using the synthetic compound [47]. Besides acting at lower concentrations the hydroxy metabolites showed much less residual inhibition 5 min after the compound was washed away, relative to DMXBA. Radioligand binding experiments also revealed higher affinities for the α7 receptor. Such affinities are much higher than shown in the *Xenopus* oocyte experiments because they are stead-ystate values probably reflective of the affinity of the desensitized state of the nicotinic receptor. While the di-hydroxy metabolite showed an efficacy (max. effect) similar to DMXBA and the monohydroxy metabolites, its electrophysiological potency and radioligand binding affinity estimates indicated less affinity for the receptor than for these other three compounds.

Two laboratories have compared the responses of human and rat α7 receptors to DMXBA and its 4OH-metabolite [9,47]. A very interesting difference has been observed by both laboratories: the efficacy of DMXBA upon the human form of the receptor is very small relative to that of the rat. However, the 4-OH metabolite displays excellent partial agonist activity on the human receptor form.

While the monohydroxy metabolites are more potent in vitro, the 4-OH metabolite at least seems less potent in vivo [29]. This may be due to poor penetration into the brain by these compounds [74,41].

Both the 4-O- and the 2-O-MXBA glucuronides have been detected [4,5,41]. The 4-O-MXBA glucuronide has been synthesized and, using the *Xenopus* oocyte, it has been shown that it displays an affinity for the rat α7 receptor similar to that of DMXBA, although its efficacy is slightly less.

Table 3
Chemical and pharmacokinetic properties DMXBA and nicotine (PK in Sprague–Dawley male rats)

Property	DMXBA [Ref.]	Nicotine [Ref.]
Ionization at pH 7.4 (%)	50 [41]	70 [23]
Partition coefficient	3810 [41]	15 [23]
Serum protein binding (%)	>80 [31]	<10 [7]
V_{ss} (l/kg)	2.2 [31]	5.2 [56]
$t_{1/2\beta}$ (h)	0.7 [31], 0.5 [5]	0.9 [56]
Oral bioavailability (%)	19 [31], 25 [5]	16 [56], 45 [7]
CL_{total} (l/h per kg)	1.4 [31]	2.90 [56]
CL_{renal} (l/h per kg)	0.022 [31]	0.20 [56]
Brain/plasma ratio	2.61 [41]	1.60 [56]

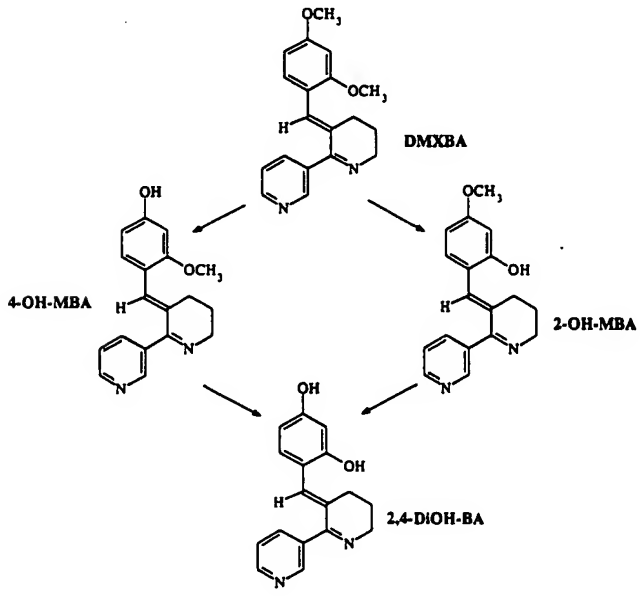

Fig. 4. Phase 1 biotransformation pathway for DMXBA [41].

6. Chronic administration effects on nicotinic recept r concentration

That chronic administration of nicotine increases the concentration of high affinity nicotinic receptors in many brain sites is well established by data obtained on experimental animals as well as upon smokers [20,42]. While there are now many papers documenting increases in receptor concentrations, fewer studies have also attempted to determine if this receptor 'up-regulation' is accompanied by a change in functional responsiveness. Most of the functional data indicate that there is a decrease in response to nicotine and related compounds which stimulate β2 subunit-containing receptors. Since tolerance to nicotine develops over time it has been tempting to utilize this 'paradoxical' or unusual increase in receptor concentration as a marker for tolerance, which is less readily measured. However, the time courses of these two alterations may not be directly linked [42].

Less is known about the effects of chronic administration of selective α7 nicotinic agonists, since these compounds have only appeared recently. Nicotine displays about 400-fold lower affinity for this receptor, so this may be one reason why changes in α7 receptor concentrations with this drug have been very small compared with the up-regulation in number of high affinity receptors. It is also difficult to measure α7 receptors by radioligand binding methods in human cortex samples due to the smaller concentrations of these receptors relatively to non-specific binding sites. Nevertheless, several studies have reported a relatively small increase in α7 concentration in the rodent brain [38,42] after in vivo administration of nicotine. Functional in vitro studies with cultured hippocampal neurons have found moderate (about 40%, maximal effect) increases in receptor concentration [6]. On the *Xenopus* oocyte expression system divergent results have been reported. In one as yet unpublished study an increase in nicotinic responsiveness as well as receptor concentration was reported [25]. A more comprehensive study comparing several nicotinic receptor subunit combinations found a very large decrease in functional α7 receptors as a result of rather brief exposures to a large nicotine pulse [52].

In several behavioral studies involving daily administration of DMXBA over a period of 2 weeks, one has failed to observe any statistically significant change in α-bungarotoxin binding (α7) or cytisine binding (predominantly α4–β2 receptor) site concentrations [3,8,72]. At the doses given, DMXBA produces robust improvements in cognitive behavior. Behavioral tolerance was not investigated. While further data is needed to assess whether receptor concentrations and tolerance occur, especially at higher concentrations, the current data suggests that α7 receptor stimulation, at least with DMXBA, does not lead to significant alterations in the receptor target which might necessitate changes in the dosage regime for treatment of Alzheimer's patients.

6.1. Preclinical in vivo toxicology of DMXBA

The intravenous LD_{50} of DMXBA for the Sprague–Dawley male rat was 16 mg/kg body weight, the i.p. LD_{50} was 68, and the per os LD_{50} was 612 [73]. The approximate i.p. LD_{50} for mice was 185 [14]. In mice, the minimal effective i.p. dose producing ataxia and hypothermia was 62 mg/kg [14]. In repeated dose studies tremors and excessive salivation were evident in some rats and dogs administered doses at least 10-fold higher than were necessary to enhance cognition. In a 26-week chronic toxicity study in dogs, there were no drug-related effects in ECG, biochemistry, hematology, opthalmology or pathology. Compared with nicotine, DMXBA had only slight or no effects on measures of cardiovascular and gastrointestinal functions [73].

6.2. Initial (phase I) clinical studies on DMXBA (GTS-21)

Thus far, the results of these studies, which were carried out in the UK, have only been reported in the form of a poster [35]. The results summarized here are from that abstract. DMXBA was administered to 87 healthy volunteers. Initially effects of single doses (range, 1–250 mg) were assessed. Subsequently subjects were orally administered (as pills) daily doses as high as 450 mg/day for 5 days. The drug was rapidly absorbed and the maximum plasma concentration (C_{max}) occurred about 1 h after administration. Both C_{max} and the area under the plasma–time curve (AUC) were proportional to dose. The elimination half-life ranged between 0.5 and 1.0 h for DMXBA and its major phase I metabolite, 3-(4-OH-2-MeO-benzylidene)-anabaseine. No serious adverse effects were reported with these doses. During the second half of the phase I tests, a Cognitive Drug Research (Reading, UK) computerized test battery was used to measure changes in cognitive function. In the single dose range 100–250 mg there was a significant effect of dose on picture recognition sensitivity and word recognition speed and delayed word recall accuracy. At twice daily doses of 75 and 150 mg for 5 days, DMXBA improved performance on these and some other measures of cognitive function in these young adult volunteers. DMXBA improved long-term memory as well as working memory and attention, as measured by the CDR test battery.

6.3. α7 receptor stimulation: some pros and cons

Interest concerning the in vivo functionality of α7 receptors has now become intense, considering that only a few years ago this receptor subtype, as measured by α-bungarotoxin binding, seemed to lack any function. Interest has been enhanced with the reported existence of this receptor not only in the central nervous system but also in the peripheral nervous system. α7 receptors have also been detected in normal as well as malignant lung cells and in T-lymphocytes. From the pharmaceutical viewpoint the α7 receptor is a desirable target since daily stimulation over time does not seem to induce changes in the receptor concentration and stimulation of the receptor does not seem to lead to drug dependence. Initial observations on the α7 knockout mouse [53] have not revealed any gross behavioral or physiological deficits. It seems likely that the α7 receptor functions primarily as a tonic modulator of physiological processes rather than as an essential step in synaptic transmission.

Another major advantage of the α7 receptor as therapeutic target is that neuroprotection by nicotinic compounds seems to be wholly mediated through this particular receptor subtype. These neuroprotective ac-

tions may ultimately provide the most benefit to AD patients if the disease can be diagnosed early enough such that neuroprotective therapy may counteract the neurodegenerative process in time. The recent finding [37] that high (30 μM) concentrations of DMXBA also can cause cell death in PC12 cells would be expected from current knowledge of the harmful effects of high concentrations of intracellular calcium, and indicate the importance of utilizing therapeutic regimes which avoid sudden and excessive elevations in intracellular calcium. Perhaps one advantage of a weak partial α7 agonist such as DMXBA is that the maximal effect is much lower and thus excessive elevations are less likely to occur.

It has been known for some time that certain strains of mice are particularly susceptible towards seizures in response to relatively high doses of nicotine. The α7 receptor has been implicated as a potential mediator of this toxic response. However, the most recent genetic studies by the Collins laboratory suggest that this receptor subtype is not a specific mediator of this convulsant action [63].

Clearly a major goal of future research and clinical testing will be to demonstrate that nicotinic agonists can provide long-term cognitive and neuroprotective benefits to individuals suffering from dementias. The initial observations, typically made over a short period of 1–2 weeks need to be extended to periods of several months. While one has focused upon the effects of nicotinic agonists in isolation, since Alzheimers is a complicated disease involving many other systems besides nicotinic receptors, it seems most likely that nicotinic agonists would be administered concurrently with drugs acting upon other sites. Assuming that acetylcholinesterase inhibitors reduce synaptic concentrations of choline, the endogenous α7 agonist [2,55], then coadministration of an α7 agonist with an AChE inhibitor would be a rational drug combination.

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